IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Orla M. Conneely et al.

Application No.: 10/620,256 Confirmation No.: 9752

Filed: July 15, 2003 Art Unit: 1656

For: PRODUCTION OF RECOMBINANT LACTOFERRIN AND LACTOFERRIN POLYPEPTIDES USING CDNA SEQUENCES IN VARIOUS ORGANISMS

Examiner: H. A. Robinson

DECLARATION UNDER 35 CFR 1.132

1. I, Atul Varadhachary, declare as follows:

2. My current position is President & Chief Operating Officer of Agennix, Inc. and Adjunct Professor of Molecular and Cellular Biology at the Baylor College of Medicine, Houston, TX. I am skilled in the art of immunology and cancer research, more specifically, the art of Lactoferrin immunotherapy in oncology. As a consequence of my research, I have a full understanding of protein expression with a focus on recombinant Lactoferrin production. See attached *curriculum vitae*.

3. I believe that one with ordinary skill in this field would have to have at least a Doctorate or its equivalent in biochemistry, genetics or related discipline, and have at least three years of experience with the recombinant expression of human lactoferrin or other proteins. Although my qualifications are greater than those required to have ordinary skill in this field, based on my work in this field and the fact that I have taught and supervised individuals with the

qualifications described above, I believe that I can provide opinions as to how one with ordinary skill would understand technical descriptions and disclosures in this field and the general knowledge that one with ordinary skill would have, as of the priority date for the patent application identified above, which is May 5, 1989.

4. I understand that the USPTO Examiner examining the above application has rejected the pending claims, in part, as not enabling one of ordinary skill in the art to practice the full scope of the claims.

5. I am submitting this declaration to demonstrate enablement of the pending claims for expressing human lactoferrin in eukaryotic cells.

6. The specification focuses on demonstrating fungal expression of lactoferrin. The success of fungal expression indicates recombinant lactoferrin expression is feasible in general and that simple transfection series with know promoter constructs would yield expression in other systems. A scientist would simply have had to substitute the appropriate expression vector and transfect the construct into these other cell types. Such variations are routine and were so at the time of this application. For example, insect cells such as the widely used *Spodoptera frugiperda* derived Sf9 cells line generally support expression of heterologous proteins capable of fungal expression. *See* Fraser MJ, Expression of eukaryotic genes in insect cultures. *In Vitro Cell Dev Biol.* 1989 Mar;25(3 Pt 1):225-35. In the discussion of a publication describing the expression of human interleukin 2 in *Spodoptera frugiperda* cells (Smith GE, Ju G, Ericson BL, Moschera J, Lahm, H-W. Modification and secretion of human interleukin 2 produced in insect cells by a baculovirus expression vector. *Proc Natl Acad Sci.* 1985 December; 82:8404-8408) the authors conclude that "In bacterial vectors, it

is usually necessary to delete the coding region for signal peptides to express the mature form of eukaryotic genes for proteins, like IL-2, with cleavable signal sequences. In contrast, IL-2 was cloned in the AcNPV expression vector with the IL-2 protein initiation site and signal peptide sequences intact. These signals were recognized in the infected insect cells and IL-2 was produced with a correct N terminus. Apparently, the signals required for signal peptide recognition and processing and protein secretion have been largely conserved between insects and mammals." Yeast systems including *Saccharomyces cervisiae* also had the ability to correctly process recombinant human proteins including those with disulphide linkages such as lactoferrin (Thim L, Hansen MT, Sorensen AR. Secretion of human insulin by a transformed yeast cell. *FEBS Lett.* 1987 February, 212(2):307-12). And successfully expression of a recombinant human protein, such as recombinant human lactoferrin in mammalian cell lines is virtually guaranteed in the case of a fungally expressible human protein. *See* Bendig MM, The production of foreign proteins in mammalian cells. *Genet Eng.* 1988;(7):91-127. Thus, following successful expression of a recombinant human protein in a fungal system, one with ordinary skill in the art would be able to use standard methods to express the recombinant human protein in a variety of other eukaryotic expression systems.

7. Co-submitted with my declaration and the accompanying response are the above-cited references describing the state of the art. These references independently substantiate my foregoing testimony.

8. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this patent, and any reexamination certificate issuing thereon.

November 01, 2006
DATE

Atul Varadhachary
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